

Self-Assembling Multidomain Peptide Hydrogels: Designed Susceptibility to Enzymatic Cleavage Allows Enhanced Cell Migration and Spreading

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Abstract: Multidomain peptides are a class of amphiphilic self-assembling peptides with a modular ABA block motif in which the amphiphilic B block drives self-assembly while the flanking A blocks, which are electrostatically charged, control the conditions under which assembly takes place. Previously we have shown that careful selection of the amino acids in the A and B blocks allow one to control the self-assembled fiber length and viscoelastic properties of formed hydrogels. Here we demonstrate how the modular nature of this peptide assembler can be designed for biological applications. With control over fiber length and diameter, gelation conditions, and viscoelastic properties, we can develop suitable materials for biological applications. Going beyond a simple carrier for cell delivery, a biofunctional scaffold will interact with the cells it carries, promoting advantageous cell–matrix interactions. We demonstrate the design of a multidomain peptide into a bioactive variant by incorporation of a matrix metalloprotease 2 (MMP-2) specific cleavage site and cell adhesion motif. Gel formation and rheological properties were assessed and compared to related peptide hydrogels. Proteolytic degradation by collagenase IV was observed in a gel weight loss study and confirmed by specific MMP-2 degradation monitored by mass spectrometry and cryo-transmission electron microscopy (cryo-TEM). Combination of this cleavage site with the cell adhesion motif RGD resulted in increased cell viability and cell spreading and encouraged cell migration into the hydrogel matrix. Collectively the structural, mechanical, and bioactive properties of this multidomain peptide hydrogel make it suitable as an injectable material for a variety of tissue engineering applications.

Introduction

Hydrogels have great potential for numerous tissue engineering and drug delivery applications due to their high water content, tissue-like viscoelastic properties, and diffusion characteristics. They are easy to handle and injectable, and cells can be encapsulated and evenly distributed in the hydrogel matrix. Natural materials such as collagen, alginate, and fibrin have been used for cell or drug delivery, but limited control over the gelation process, mechanical properties, and degradation kinetics have led to modifications, combinations, and the development of synthetic hydrogels.¹ Whereas previously these materials were utilized as bioinert, passive vehicles to temporarily provide structural support to cells, hydrogels are now used to create bioactive materials which display biological cues that promote specific cell–matrix interactions. The overarching goal is to generate scaffolds with close structural and biochemical similarity to the cells' natural environment, the extracellular matrix (ECM).

The ECM mainly consists of a fibrous network of collagen, which provides tensile strength and support to cells. Elastin enables stretch and recoil, and proteoglycans bind water, supply compression resistance, and sequester growth factors. Various noncollagenous proteins orchestrate the cells' dynamic behavior such as adhesion, migration, and differentiation. The cells receive cues from the ECM, but at the same time, they actively secrete, interact with, and constantly remodel their environment, thus reinforcing their phenotype. By excreting proteases, mainly plasmin or matrix metalloproteinases (MMPs), cells can degrade the matrix around them and replace it with newly synthesized ECM proteins. This process in turn affects cellular behavior, as it has been found that MMPs are associated with proliferation, migration, apoptosis, and differentiation during development, tissue remodeling, and angiogenesis. However, MMPs also play a role in pathological processes and cancer formation.² The MMP-2 gene encodes an enzyme that degrades collagen IV, the type of collagen present in basement membrane, and also has the ability to degrade several other types of collagen including fibrillar collagen type I, the main component in extracellular matrix of connective tissues.³

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Although ECM mimics that reproduce all ECM characteristics have yet to be developed, a number of attractive materials with key ECM properties have been described.^{4–13} Recently, our laboratory has developed an amphiphilic, self-assembling peptide system we call multidomain peptides (MDP) due to their modular design.^{14,15} The MDPs are designed to display distinct regions of function or “domains”, arranged in an ABA block motif, and self-assemble into nanofibers 6 nm in diameter with design-dependent length. The process of supramolecular assembly is driven by a core motif (the B block) of alternating hydrophilic and hydrophobic amino acid residues. In an aqueous environment, the side chains segregate to opposing sides of the peptide backbone, creating a facial amphiphile. Two of these peptides can then form a “sandwich” stabilized by hydrophobic packing between leucine residues. Fiber elongation occurs as the dimers string together through antiparallel β -sheet hydrogen bonding oriented down the fiber axis. Charged amino acid residues in the flanking A region provide water solubility and also work against fiber assembly via electrostatic repulsion and can thus be utilized to control fiber assembly and length.¹⁴ For example, addition of oppositely charged multivalent ions can screen these charges and result in physical cross-linking, fiber elongation, entrapment of water, and gelation.¹⁵

With a fiber diameter of 6 nm, nanofibers created from MDPs mimic the nanoscale dimensions and structure of natural ECM, where cells can bind to the fibers via adhesion molecules but still interact with other cells.¹⁶ True three-dimensional cell growth within the nanofibrous gels make these materials promising candidates as scaffolds for cell delivery. Incorporation of bioactive sequences in either the central block or the flanking regions of MDPs may enhance cell–matrix interactions and promote desired cellular responses. However, the organization of MDP molecules into organized β -sheet aggregates with a fiber assembly process and structure reminiscent of amyloid raises concerns that the material may not be degradable in vivo and therefore might not be suitable as a tissue engineering scaffold. This is underscored by the devastating effects of amyloid aggregates observed in Alzheimer’s disease. By programming susceptibility to proteolytic degradation into our β -sheet-forming peptides, MDPs may be adapted to a variety of biological applications. Based on the general ABA block structure of multidomain peptides as exemplified in $K_2(SL)_6K_2$ (MDP 1, see Table 1),¹⁵ a hexapeptide containing the MMP-2

Table 1. Names and Sequences of Peptides Studied

MDP #	Sequence
1	KK-SLSLSLSLSLSL---KK
2	KK-SLSLSLSLSLSL---KKGRGDS
3	KK-SLSLSLRGSLSLSL-KK
4	K-SLSLSLRGSLSLSL-K
4a	K-SLSLS
4b	LRGSLSLSL-K
5	K-SLSLSLRGSLSLSL-KGRGDS

consensus cleavage motif LRG¹⁷ in the central block motif was designed. We show that this modification results in susceptibility to degradation with collagenase IV and, to a lesser extent, trypsin by gel weight loss over time. Additionally, specific degradation with MMP-2 was observed by mass spectrometry and cryo-transmission electron microscopy (cryo-TEM). Incorporation of both the cleavage site and the well-known three amino acid cell adhesion motif RGD¹⁸ made this variant compatible for cell culture, leading to good cell spreading, proliferation, and increased migration into the assembled hydrogel.

Experimental Section

Peptide Synthesis. All peptides were synthesized by solid-phase chemistry on an Advanced Chemtech Apex 396 peptide synthesizer using a protocol described previously¹⁵ that had been optimized for strongly β -sheet-forming peptides. After acylation of the N-terminus and cleavage from the resin, the crude peptides were dissolved in deionized water at 5 mg/mL and dialyzed in semipermeable membranes with a molecular weight cutoff of 100–500 Da (Spectra/Por, Spectrum Laboratories Inc., Rancho Dominguez, CA). The water was changed every 12 h for 5 days, the peptides were lyophilized, and the resulting products were used for further analysis and cell culture experiments. The correct masses of all peptides were confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (see Supporting Information).

Gel Formation and Rheological Properties. The lyophilized peptides were dissolved in deionized water containing 298 mM sucrose at 20 mg/mL, and the pH was adjusted to 7.4. Gelation was induced by addition of phosphate buffer, where lysine-containing peptides are cross-linked due to the presence of negatively charged phosphate ions, to a final peptide concentration of 10 mg/mL (1% by weight). To evaluate viscoelasticity and gelation behaviors of MDP hydrogels, oscillatory stress sweep analysis was performed 24 h after induction of gelation (AR-G2, TA Instruments, 8 mm parallel steel plates). Aliquots (50 μ L) of gel were pipetted onto the center of the plate and a gap of 250 μ m was established. Storage modulus (G') and loss modulus (G'') were measured as a function of oscillatory stress ranging from 0.01 to 1000 Pa at an angular frequency of 0.5 rad/s.

Weight Loss. To determine the degradation profile of MDPs with and without an MMP-2 cleavage sequence, MDP 1 and 4 hydrogels were prepared as described above, and for each sample, 50 μ L of gel was transferred to a centrifuge tube after the empty tube was weighed. After 30 min of equilibration, 200 μ L of phosphate-buffered saline (PBS) was added on top of each gel, and samples were stored at 37 °C for 2 h. The PBS solution was then removed, and the weight of each gel was determined. Samples were divided into three groups and incubated with 200 μ L of (1) collagenase IV (Sigma–Aldrich, St. Louis, MO) at 3 mg/mL in PBS or (2) trypsin 0.05% with tetrasodium ethylenediaminetetraacetate (Na₄EDTA; Invitrogen, Carlsbad, CA) or (3) PBS as a

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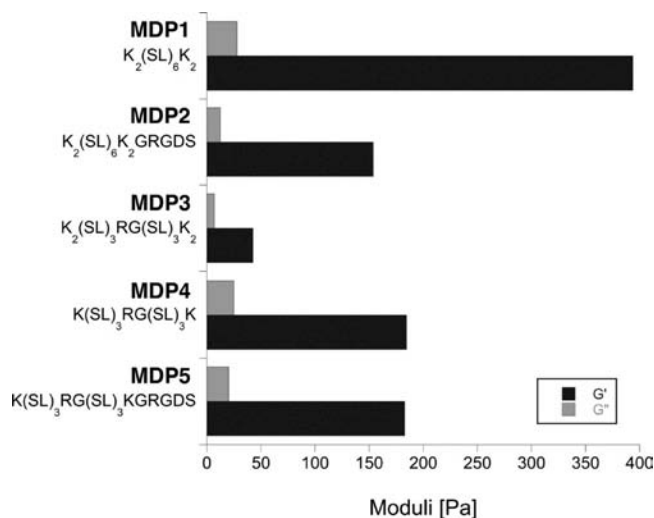


Figure 1. Storage (black) and loss moduli (gray) of MDPs 1–5 containing variants with and without cleavage sequence, cell adhesion motif, and variable-length flanking region.

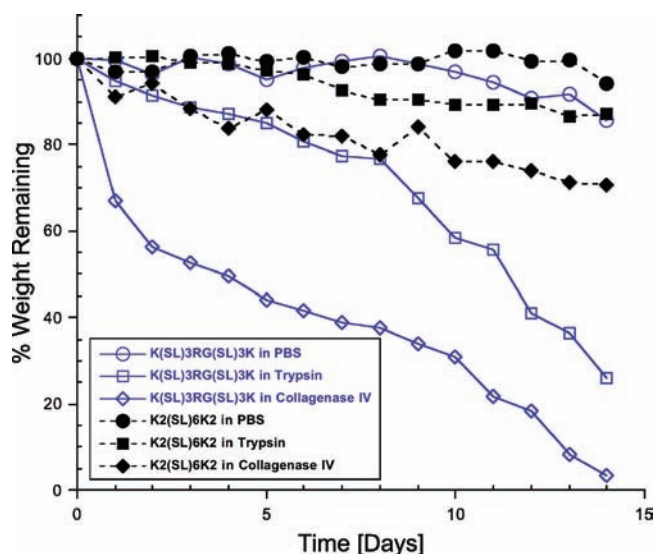


Figure 2. Hydrogels from MDP 4 (blue open circles) and MDP 1 (black solid circles) as a control were incubated with trypsin (squares) or collagenase IV (diamonds) and compared to a negative control in PBS. The weight of the hydrogels was determined each day. Data points show averages of three samples. Weight loss is depicted as a percentage where the weight at the beginning of the experiment is 100%.

negative control. Samples were run in triplicate, solutions were changed, and the sample weight was determined every 24 h for 14 days.

MMP-2-Specific Cleavage. To assess MMP-2-specific cleavage, gels of MDP 1, 4, and 5 were prepared at a concentration of 20 mg/mL and then mixed with MMP-2 (Sigma–Aldrich, St. Louis, MO) in PBS, with the final concentrations being 10 mg/mL peptide and 100 ng of MMP-2. Samples were incubated at 37 °C for 48 h, and enzymatic cleavage was assessed by mass spectroscopy. For MDP 4, digestion was also visualized by vitreous ice cryo-TEM. For cryo-TEM analysis, a small quantity of the sample solution (2–3 μ L) was applied to a TEM copper grid with holey carbon film purchased from Quantifoil (400 mesh Cu grid, 1.2 μ m hole diameter), and blotted with filter paper by use of a Vitrobot type FP 5350/60 under 100% relative humidity for 2 s to create a thin layer of sample on the surface of the grid. The grid was plunged into liquid ethane and quickly transferred to liquid nitrogen. Samples

were analyzed on a JEOL 2010 TEM at an accelerating voltage of 200 kV under low-dose imaging conditions.

Cell Proliferation and Migration. To assess the compatibility of the newly created peptide hydrogel with cell cultures, MDP 1 and the peptide containing the cleavage sequence, MDP 4, were modified to include the cell adhesion motif RGDS at the C-terminus, resulting in MDP 2 and 5. For cell viability and migration studies, mesenchymal stem cells from human exfoliated deciduous teeth (SHED) were used,¹⁹ which were kindly provided by Dr. Songtao Shi at USC. The cells were cultured in α -MEM (modified Eagle medium) supplemented with 15% fetal bovine serum, 50 μ g/mL L-ascorbic acid 2-phosphate, 100 units/mL penicillin, and 100 μ g/mL streptomycin and incubated at 37 °C with 5% CO₂. Subconfluent cells of passage 5 were detached by use of trypsin–EDTA (Invitrogen, Carlsbad, CA), and cells were encapsulated at a density of 1.0×10^5 per gel. Hydrogels were prepared at 20 mg/mL and mixed with a cell suspension in PBS to create gels with a final peptide concentration of 10 mg/mL. Gels containing SHED were seeded in 96-well plates with a gel volume of 100 μ L, and 200 μ L of medium was added on top of each gel 30 min later. Culture medium was changed every other day, and MTT assay for cell viability was performed after 3, 7, and 14 days of incubation as follows: Gels were incubated with 200 μ L of medium without serum containing 2 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma–Aldrich, St. Louis, MO) for 3 h. The solution was removed, and gel and cells were lysed in 200 μ L of dimethyl sulfoxide (DMSO; Sigma–Aldrich, St. Louis, MO); the plates were shaken thoroughly for 5 min. Absorbance was measured in a 96-well plate reader at 570 nm against a blank reading prepared from gels without cells. Five samples were seeded per experiment. Two independent experiments were performed, resulting in a sample size of 10. For statistical analysis, the nonparametric Kruskal–Wallis test was performed to determine significant differences in cell viability between the control (MDP 1) and modified peptides (MDP 2, 4, and 5).

To determine whether the presence of the enzyme-cleavable site would encourage cell migration into the hydrogels, SHED was incubated with membrane-permeable fluorescein diacetate (Cell Tracker Green, CMFDA; Invitrogen, Carlsbad CA) for 30 min and then washed with PBS, detached, and seeded on top of hydrogels of MDP 1 or the cleavage peptide MDP 4. Cells were seeded on top of preformed gels at a density of 5×10^4 cells, which were allowed to adhere for 24 h. Samples were fixed in 2% paraformaldehyde and embedded for cryosectioning after 1 and 5 days in culture. Sections of 10 μ m thickness were prepared on a cryostat microtome, mounted on slides, and stored at –20 °C. Before use, cells were permeabilized with 5% Triton X in PBS, and cell nuclei were stained with DAPI (4',6-diamidino-2-phenylindole). Cells and hydrogels were visualized by use of a Zeiss LSM 510 Meta confocal microscope with an attached photomultiplier tube (PMT).

Results and Discussion

Peptide Design. Previously we have shown that $K_2(SL)_6K_2$ (MDP 1) has excellent self-assembly and gelation characteristics, making good nanofibers with a diameter of approximately 6 nm and gels that undergo shear thinning and recovery.¹⁵ This peptide design was modified to create four different variants with an MMP-2-specific cleavage motif, an RGDS adhesion sequence, and either one or two lysine residues in the flanking regions (see Table 1).

Specificity of enzymatic cleavage by endopeptidases arises from the amino acid sequence. Occurrence of specific residues 5 positions N-terminal and 3 positions C-terminal from the scissile bond (P5' and P3) has been demonstrated to affect recognition and increase the specificity of an enzyme for its substrate.¹⁷ The consensus MMP motif LRG (leucine-arginine-glycine) was chosen on the basis of cleavage site motifs

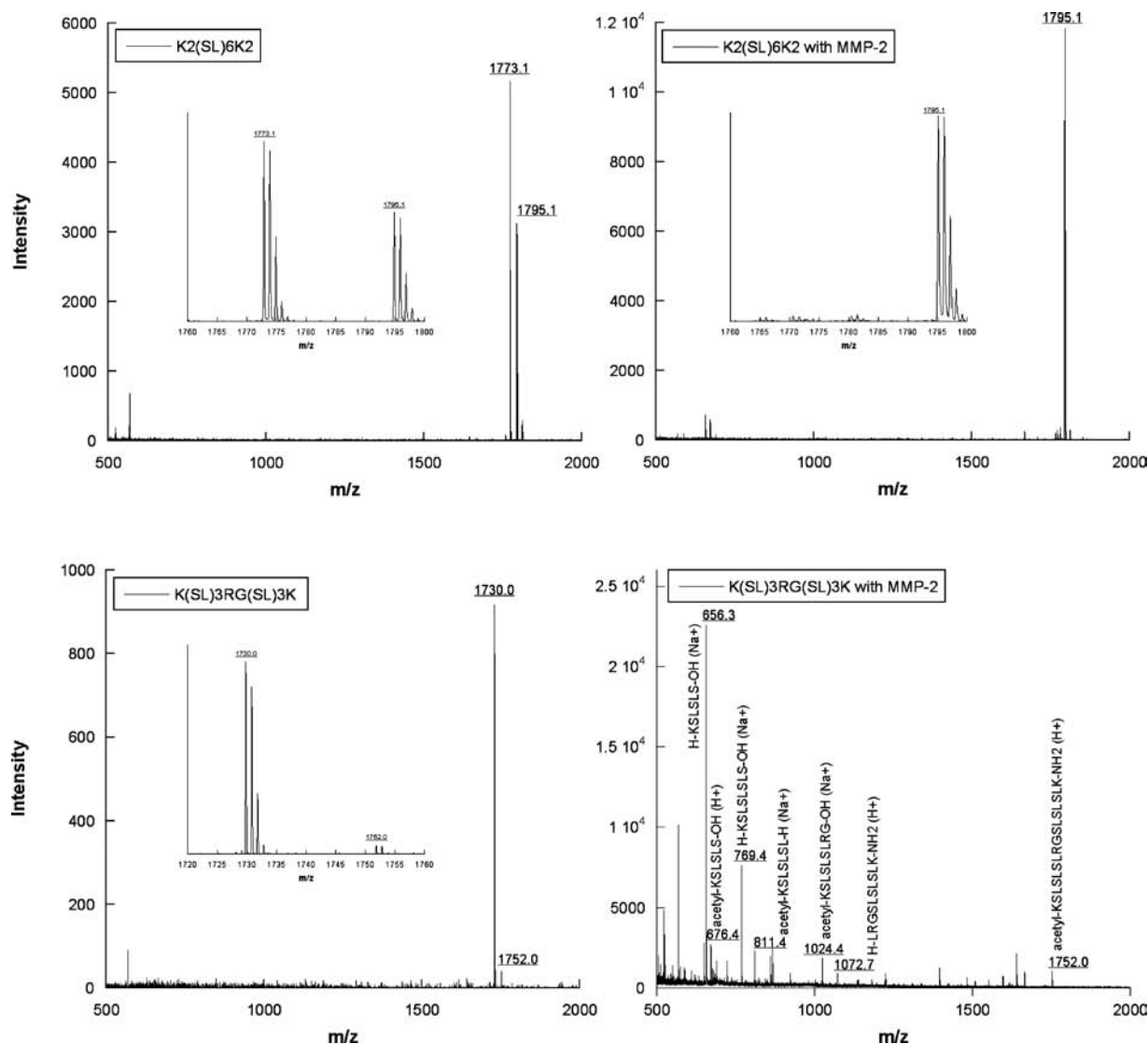


Figure 3. MALDI-TOF mass spectrometry of MDP **1** and **4** before and 48 h after incubation with MMP-2. Whereas the control peptide shows a single peptide before and after digestion, the cleavage peptide is degraded and multiple fragments can be observed. The expected fragments after cleavage, KSLSL and LRGSLSLK, are both present. Additional fragments could be identified as alternative cleavage products, whereas some of the peaks remained unidentified.

described previously.¹⁷ The sequence SLSLRG was conveniently incorporated into the central block with modification of only two amino acids of MDP **1**. In order to compensate for what was expected to be an unfavorable influence on self-assembly and gel formation of the arginine residue on the hydrophilic face of the peptide and glycine on the hydrophobic face, the amphiphilic B block was elongated by one (SL) repeat.

After assessment of solubility, gelation properties and confirmation of enzymatic cleavage of these peptides, MDP **4** was chosen for further cell culture studies. Along with MDP **1**, these peptides were synthesized with the cell adhesion motif RGD, resulting in MDPs **2** and **5**. An N-terminal glycine was added as a spacer, and a C-terminal serine was added to increase the specificity of this motif.²⁰ The expected fragments after enzymatic cleavage were also synthesized (MDP **4a** and **4b**) to assess their structural characteristics, particularly the possibility that they could still assemble and form β -sheet fibers or gels.

Rheological Properties. The storage modulus G' and the loss modulus G'' were determined for all MDPs by oscillatory stress sweep analysis (Figure 1). G' for MDPs **2** and **3** were found to

be significantly lower than for MDP **1**. In the case of MDP **3** ($G' = 43$ Pa) the handling properties were poor enough that it would be impractical to use, as we have found that hydrogels with storage modulus below 80 Pa are too weak to survive even gentle handling such as washing and exchanging of buffers. To resolve this problem, the flanking region of lysine repeats were shortened in MDP **4** and **5**. The reduced electrostatic repulsion from these amino acids was expected to result in an improved G' , and this was in fact observed to be the case as MDP **4** and **5** have storage moduli of just over 175 Pa at 1% by weight. This illustrates the modular nature in which this class of peptides can be designed and modified. The data presented here refer to peptide gels of 1% by weight, but substantially stronger gels can easily be produced by increasing the peptide concentration.

Degradation of Peptides with Enzyme-Cleavable Site. Figure 2 shows the weight loss of MDP **4** compared to the parent MDP **1**. For both, incubation with PBS resulted in a weight loss of 5–10% over a 2-week period. This amount of loss is probably due to small amounts of peptide that are washed off due to daily changes of the supernatant. Incubation with collagenase IV

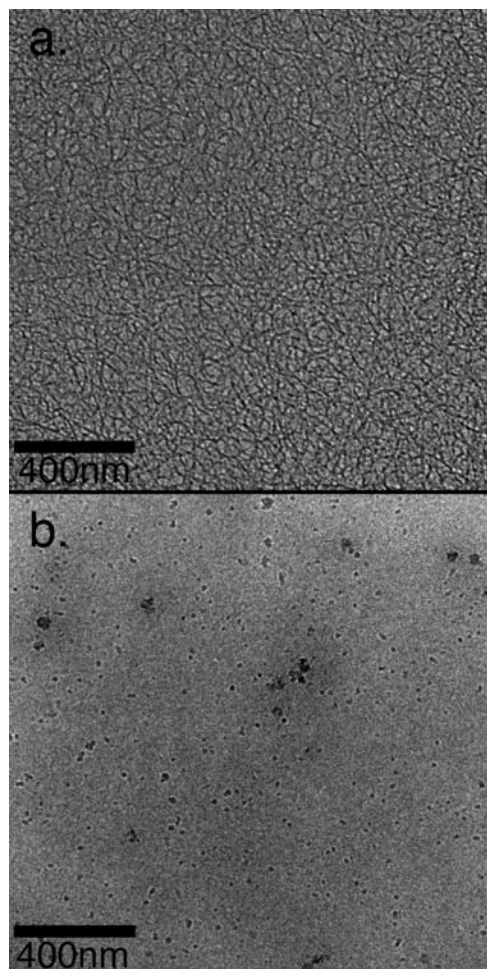


Figure 4. Cryo-TEM images (a) before and (b) after incubation with MMP-2. The nanofibers disintegrate, leaving small aggregated structures that do not cross-link.

resulted in complete digestion of MDP 4 after 14 days, whereas the control peptide was reduced by only 30% of its original weight. Whereas a lower storage modulus of MDP 4 compared to MDP 1 may be a contributing factor to its more rapid weight loss, we believe the major difference is due to its enzyme cleavage site. This is based on observations of a similar peptide (data not shown) with a cleavage site and a storage modulus well below that of MDP 4 (68 Pa vs 185 Pa), yet its degradation rate was nearly identical. This peptide was later eliminated from this study due to its low mechanical stability.

Trypsin can also be used to digest the peptide, although at a slower rate than collagenase IV, where 75% of the gel is degraded after 2 weeks. This rather unspecific protease present in the digestive tract cleaves C-terminally of lysine or arginine and is commonly used to detach adherent cells from tissue culture plates. The effect of hydrogel degradation by trypsin might be interesting for cell culture applications, where entrapped cells could be separated from the gel. Mechanical disruption and dilution of hydrogels in trypsin solution accelerates the digestion process dramatically and enables harvesting live cells for further experiments.

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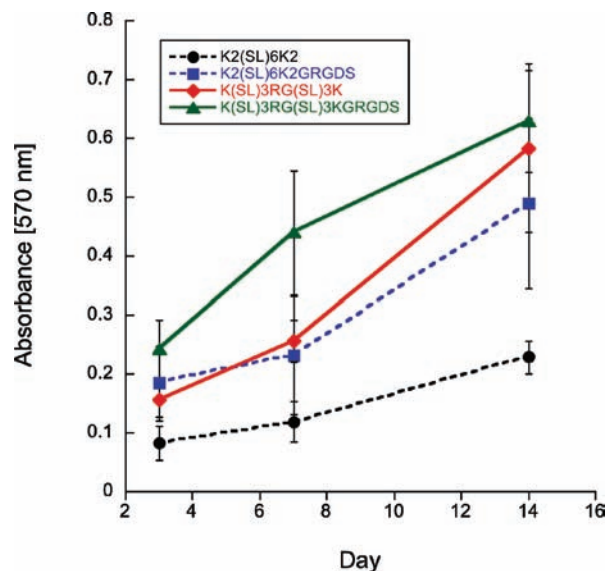


Figure 5. Cell viability in MDP 1, 2, 4, and 5 (black circles, blue squares, red diamonds, and green triangles, respectively) as determined by MTT assay after 3, 7, and 14 days in culture. Symbols and bars represent mean values and standard deviations ($n = 10$). Viability is significantly lower in the unmodified peptide; the presence of the cell adhesion motif and the cleavage site increase cellular activity, and the combination of both in MDP 5 results in highest viability and indicates that this is the most favorable environment for cell proliferation among these four peptide hydrogels.

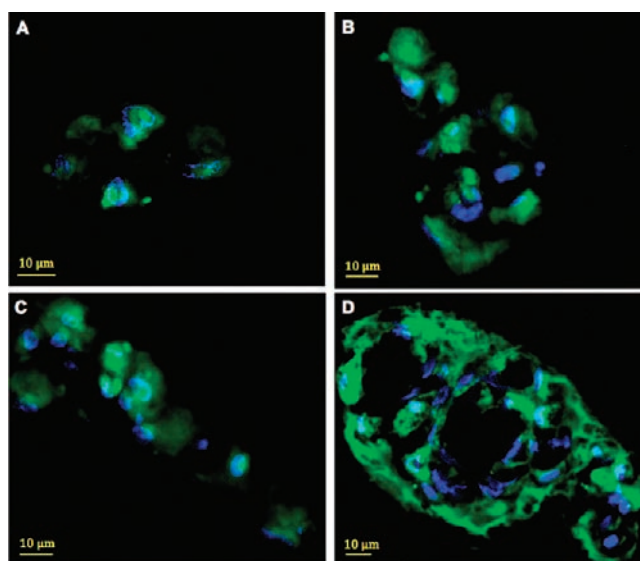


Figure 6. Fluorescently labeled cells visualized by confocal microscopy (magnification $63\times$) barely spread in the parent peptide (MDP 1, panel A), but they show enlarged cell bodies when either the cell attachment motif (MDP 2, panel B) or the cleavage site is present (MDP 4, panel C). Combination of both bioactive peptide motifs results in enhanced cell spreading (MDP 5, panel D).

Upon mixing a 2 wt % solution of MDP 1, 4, or 5 with MMP-2 in PBS at a ratio of 1:1, hydrogels form, but MDPs 4 and 5, which contain the enzyme cleavage site, are degraded after 48 h of incubation at 37 °C. This rapid degradation is in contrast to what is observed if the enzyme is merely placed on top of a gel and is likely due to the much higher area of contact obtained by the mixing process. MDP 1 is not affected. MALDI-TOF mass spectroscopy was performed on the peptide mixtures (Figure 3). MDP 1 shows the expected full-length peptide peak before as well as after incubation with MMP-2. In contrast, the

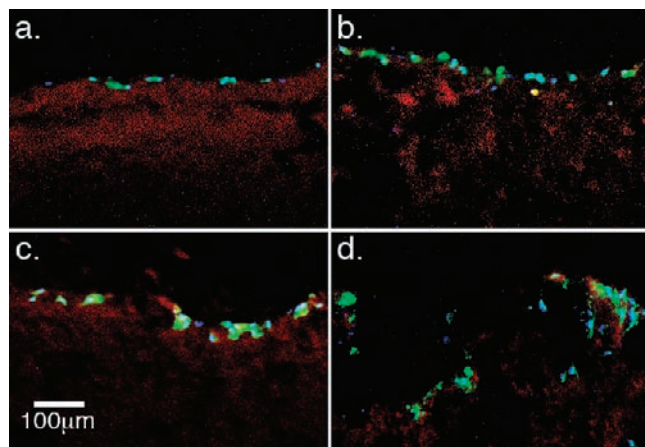


Figure 7. Green-fluorescent cells were seeded on top of MDP **2** without cleavage site (a, b) and MDP **5**, where the cleavage motif is present (c, d). Images show cells after 1 day (a, c) and after 5 days (b, d) in culture. Whereas cells remain as a monolayer on top of MDP **2** hydrogels, they migrate into MDP **5**.

cleavage peptide shows one peak corresponding to the mass of the peptide molecule before incubation but many fragments afterward. For MDP **4**, the peak with the highest intensity could be identified as the N-terminal fragment KSLSLK; the C-terminal fragment, LRGSLSLK, is also observed as a small peak. Several additional species were present, many of which corresponded to smaller peptide fragments present in the mixture. The diversity of peptides observed may be attributed to the fact that, after cleavage into the two main fragments, the smaller peptide molecules are susceptible to further degradation. Some of the peaks could not be identified as cleavage fragments and might be due to nonspecific digestion of the enzyme itself.

Degradation of MDP **4** nanofibers was visualized by cryo-TEM as illustrated in Figure 4. Whereas MDP **1** remains intact after incubation with MMP-2, MDP **4** transforms from a dense fibrous network to amorphous aggregated remnants, suggesting cleavage and subsequent disintegration of the hydrogel. Neither MDP **4a** nor **4b**, which are analogous to the expected N- and C-terminal cleavage fragments, formed gels. This indicates that the truncated peptides after degradation of the starting material are no longer able to assemble and form insoluble aggregates, which is a critical point for any *in vivo* applications. Additionally, neither of these fragments displayed a CD spectrum characteristic of a β -sheet but instead displayed a simple random coil signature (see Supporting Information).

Two different procedures were used to characterize the profile of enzymatic digestion. Addition of collagenase IV on top of the hydrogels resulted in a degradation profile showing continuous mass loss, indicating surface erosion of the material. For bulk degradation, an initial plateau phase would be expected, followed by a rapid drop of the curve. Surface degradation is the preferred mechanism for many biological applications, as it allows migrating cells to locally degrade the matrix as they migrate while overall mechanical integrity of the scaffold is maintained until sufficient amounts of ECM have been produced to replace the synthetic material.

Mixture of hydrogels with MMP-2 results in more rapid disruption of the fibrous network, as can be expected due to the increased surface area for the enzyme to attack and higher specificity of MMP-2. Crude collagenase contains a varying mixture of enzymes and digests a wider range of substrates. The results after incubation with collagenase IV therefore show

proteolytic susceptibility of variant MDP, whereas the digestion with MMP-2 confirms specificity of this enzyme toward the substrate we created. This series of experiments confirms that specific digestion of MDPs occurs when required amino acid residues are present in certain positions relative to the scissile bond: a hydrophobic amino acid at P1' followed by a basic amino acid and a small residue.

The degradation rate of the peptide hydrogels in a biological setting will depend on many variables, including cell seeding density, susceptibility of the cleavage site, amount of the targeted matrix metalloproteinase synthesized by the cells, and presence of growth factors. In order to adjust cell-mediated matrix degradation and cellular ECM production to ensure sufficient and necessary support of cells by the synthetic matrix, both parameters need to be monitored under a variety of conditions. For example, faster degradation could be induced by higher cell numbers, and slower degradation could be achieved by incorporation of peptides with cleavage sites for other MMPs, which are expressed in smaller quantities in the targeted cells or tissues.

Following these experiments, MDP **4**, which has the same number of amino acids as the parent peptide MDP **1**, is susceptible to enzymatic cleavage, and is able to form stable hydrogels at physiological pH, was chosen for further analysis as well as modification for cell culture studies.

Influence of the Cleavage Site on Cell Proliferation and Spreading. In order to investigate the compatibility of the parent and variant peptides with living cells, both MDP **1** and **4** were modified and synthesized with the cell adhesion motif RGD at the C-terminus. Viability was enhanced in MDP **2**, **4**, and **5** (which contain either the adhesion motif, the enzymatic cleavage site, or both, respectively) compared to MDP **1** (Figure 5). This difference was significant for all time points (days 3, 7, and 14). The highest metabolic activity and viability was observed for cells encapsulated in MDP **5**, suggesting that the presence of an enzyme-cleavable site along with the cell adhesion motif creates the most favorable environment for cells.

The benefit of both adhesion sequence and cleavage sequence was more strongly demonstrated visually after encapsulation of green fluorescent cells into the hydrogel. Whereas spreading could barely be observed in MDP **1** hydrogels, cell morphology changed slightly in MDP **4**, visibly in MDP **2**, but the biggest change could be observed in MDP **5**, where the combination of cleavage site and cell adhesion motif seem to have a synergistic effect on cell spreading (Figure 6). This is in accordance with previous work, which suggested that initial cell spreading is dependent on proteolytic susceptibility²¹ of the matrix. The presence of an MMP-2 cleavage site also dramatically influenced cell migration into MDP hydrogels. When SHED were seeded on top of the gels created from MDP **1** versus **4**, cells migrated into the MDP **4** hydrogel to a much greater extent (Figure 7).

In conclusion, incorporation of an MMP-2-specific cleavage motif in β -sheet-forming peptides resulted in enzyme-mediated digestion and collapse of the hydrogel due to disruption of the nanofibrous network. Furthermore, we demonstrate an immediate effect of the peptide design on cellular behavior, where incorporation of the cleavage site markedly enhanced cell viability, spreading, and migration. This specific interaction of cells with the synthetic matrix surrounding them is another step toward the development of ECM mimics. The versatility of MDPs leaves a variety of possibilities for further modifications and design of custom-made scaffolds for regenerative medicine.

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Supporting Information Available: Additional CD and MALDI-TOF MS analysis. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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